

Figure S1. Comparison of transcript levels of genes located on chromosomes 12q14.1 and 19q13.32 between poor and good responders. Differential analysis revealed higher *CDK4* transcript levels in poor responders that were associated with gain of chromosome 12q14.1. AGAP2, ArfGAP with GTPase domain, ankyrin repeat and PH domain 2; TSPAN31, tetraspanin 31; MARCH9, membrane-associated ring-CH-type finger 9; CYP27B1, cytochrome P450 family 27 subfamily B member 1; BCL3, BCL3 transcription co-activator; CBLC, Cbl proto-oncogene C; BCAM, basal cell adhesion molecule; PVRL2, poliovirus receptor-related 2; TOMM40, translocase of outer mitochondrial membrane 40; APOE, apolipoprotein E; Y, yes; N, no; ChOS, Chiba osteosarcoma.

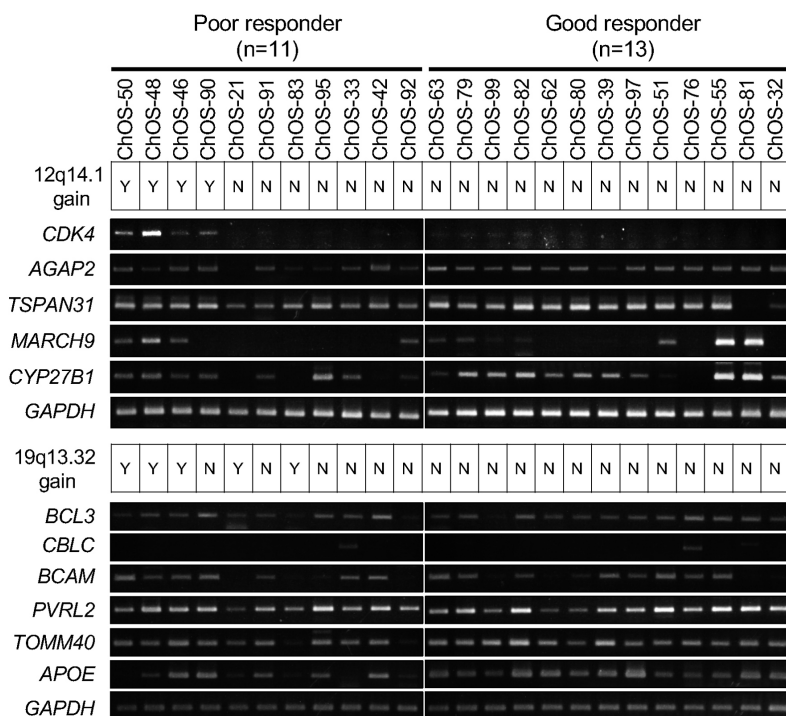


Table SI. Clinical information of 50 patients with primary OS.

ChOS-no.	Age at diagnosis, years	Sex	Location	Metastasis at diagnosis	Stage	Subtype	Surgery	Surgical margin	Chemotherapy regimen	Chemosensitivity
3	8	Male	Femur	No	2B	Osteoblastic	Resection	Wide	MAP-I	Good
5	15	Male	Femur	Yes	4A	Fibroblastic	Amputation	Wide	MAP-I	Good
13	12	Female	Tibia	No	2B	Osteoblastic	Resection	Wide	MAP	Poor
17	24	Male	Femur	No	2B	Osteoblastic	Resection	Wide	MAP-I	Poor
18	21	Female	Tibia	No	2A	Osteoblastic	Resection	Wide	MAP-I	Good
21	14	Female	Femur	No	2B	Chondroblastic	Resection	Wide	MAP	Poor
23	15	Male	Femur	No	2A	Osteoblastic	Resection	Wide	MAP	Good
25	10	Male	Femur	No	2B	Osteoblastic	Resection	Wide	MAP	Good
26	10	Female	Humerus	No	2A	Chondroblastic	Resection	Wide	MAP-I	Good
27	11	Female	Femur	No	2B	Osteoblastic	Resection	Wide	MAP	Good
31	13	Male	Femur	No	2A	Osteoblastic	Resection	Wide	MAP	Good
32	17	Female	Ulna	Yes	4A	Osteoblastic	Resection	Wide	MAP	Good
33	6	Male	Tibia	No	3	Osteoblastic	Amputation	Wide	MAP-I	Poor
34	23	Male	Tibia	No	2A	Osteoblastic	Resection	Marginal	MAP-I	Poor
36	19	Female	Femur	No	2A	Osteoblastic	Resection	Wide	MAP-I	Poor
38	21	Female	Femur	No	2B	Osteoblastic	Resection	Wide	MAP-I	Poor
39	18	Male	Radius	No	2A	Osteoblastic	Resection	Wide	MAP	Poor
40	16	Female	Fibula	No	2A	Osteoblastic	Resection	Wide	MAP-I	Good
41	9	Male	Tibia	No	2A	Osteoblastic	Amputation	Wide	MAP-I	Good
42	20	Male	Tibia	No	2B	Osteoblastic	Resection	Wide	MAP-I	Poor
45	29	Female	Tibia	No	2A	Osteoblastic	Resection	Wide	MAP-I	Poor
46	10	Female	Femur	No	2B	Osteoblastic	Resection	Wide	MAP	Poor
48	8	Male	Femur	No	2B	Osteoblastic	Amputation	Wide	MAP-I	Poor
49	18	Male	Fibula	Yes	4A	Osteoblastic	Resection	Wide	MAP-I	Poor
50	10	Male	Femur	No	3	Chondroblastic	Resection	Wide	MAP-I	Good
51	20	Female	Tibia	No	3	High-grade surface	Amputation	Wide	MAP-I	Poor
52	12	Male	Femur	No	2A	Osteoblastic	Resection	Wide	MAP	Good
54	17	Female	Femur	No	2A	Fibroblastic	Resection	Wide	MAP-I	Good
55	20	Male	Femur	No	2B	Osteoblastic	Resection	Wide	MAP	Poor
56	21	Male	Tibia	No	2A	Osteoblastic	Resection	Wide	MAP	Good
58	19	Male	Femur	No	2B	Osteoblastic	Resection	Wide	MAP	Good
62	12	Male	Femur	No	2B	Osteoblastic	Amputation	Wide	MAP-I	Good
63	20	Male	Femur	No	2B	Osteoblastic	Resection	Wide	MAP-I	Good
76	15	Male	Tibia	No	2A	Fibroblastic	Resection	Wide	MAP-I	Good
79	16	Male	Femur	No	2B	Osteoblastic	Resection	Wide	MAP-I	Good
80	16	Male	Femur	No	2B	Osteoblastic	Resection	Wide	MAP	Good
81	10	Female	Femur	No	2A	Osteoblastic	Resection	Wide	MAP	Good

Table SI. Continued.

ChOS-no.	Age at diagnosis, years	Sex	Location	Metastasis at diagnosis	Stage	Subtype	Surgery	Surgical margin	Chemotherapy regimen	Chemosensitivity
82	13	Male	Femur	No	2A	Osteoblastic	Resection	Wide	MAP	Good
83	10	Male	Humerus	Yes	4A	Osteoblastic	Resection	Wide	MAP-I	Poor
84	21	Male	Femur	No	2B	Osteoblastic	Resection	Wide	MAP-I	Poor
85	11	Male	Tibia	No	2A	Osteoblastic	Resection	Wide	MAP	Poor
90	11	Male	Femur	No	2B	Osteoblastic	Resection	Marginal	MAP	Poor
91	11	Male	Femur	Yes	4A	Osteoblastic	Resection	Wide	MAP	Poor
92	13	Male	Femur	No	2B	Osteoblastic	Resection	Wide	MAP	Poor
95	21	Male	Femur	Yes	4A	Osteoblastic	Resection	Wide	MAP	Poor
97	17	Male	Femur	No	2B	Osteoblastic	Resection	Wide	MAP	Good
99	7	Female	Tibia	No	2A	Osteoblastic	Resection	Wide	MAP-I	Good
104	18	Female	Tibia	No	2B	Osteoblastic	Resection	Wide	MAP	Good
106	8	Female	Femur	No	2B	Chondroblastic	Resection	Wide	MAP	Good
108	15	Male	Fibula	No	2B	Osteoblastic	Resection	Marginal	MAP	Poor

ChOS, Chiba osteosarcoma; MAP, methotrexate, adriamycin and cisplatin.

Table SII. PCR primers.

Gene	Forward, 5'→3'	Reverse, 5'→3'
CDK4	GACTGGCCTCGAGATGTATC	GTAGATAAGAGTGCTGCAGAGC
AGAP2	AGCATTCGAGAGGCTGTGAT	AAGACCCGATCCACATTGAG
TSPAN31	GCCCCTTGTCACATCCTCTA	GAGCCACACCAGGGTATGTT
MARCH9	CTCCTCCCACTCATCCAGAG	TGCAACCTGTGCAGAGAAAC
CYP27B1	GACTCAGGAAGCCAGGTGAG	GACGAAGGACCAACCAGGTA
BCL3	AACACCGAGTGCCAAGAAAC	TGTCGTTGTGGCAGTTCTTG
CBLC	TATGTGAGCTCAGATGGCAG	CACGGCTCAATCTTCACATC
BCAM	TGAATCTCGAGGGGAACTTG	AGGTAAGGAAAGCACCTTCC
PVRL2	AAGACCCCCTACTTTGATGC	GGGACATGACAAAGCCTTTG
TOMM40	ACCAGTACGAGTCGAAGTTC	TGTGGTTGACCTGAAAATGG
APOE	GGCCTACAAATCGGAAGTGG	CGGCGCTTCTGCAGGTCATC
GAPDH	ACCTGACCTGCCGTCTAGAA	TCCACCACCCTGTTGCTGTA

AGAP2, ArfGAP with GTPase domain, ankyrin repeat and PH domain 2; TSPAN31, tetraspanin 31; MARCH9, membrane-associated ring-CH-type finger 9; CYP27B1, cytochrome P450 family 27 subfamily B member 1; CBLC, Cbl proto-oncogene C; BCAM, basal cell adhesion molecule; PVRL2, poliovirus receptor-related 2; TOMM40, translocase of outer mitochondrial membrane 40; APOE, apolipoprotein E.

Table SIII. Non-synonymous somatic mutations identified by targeted exome sequencing of 16 primary OS tumors using 409 cancer-associated gene panels.

ChOS no.	Chemosensitivity	CDK4 gain	Total SNV	Non-synonymous SNV	Locus	Genotype	Gene	Location	Coding	Amino acid change	Variant effect	Allele ratio
48	Poor	Yes	4	2	chr6:52880907	G/A	<i>ICK</i>	Exonic	c.805C>T	p.Pro269Ser	Missense	G=0.57, A=0.43
50	Poor	Yes	7	4	chr14:102548595	G/A	<i>HSP90AA1</i>	Exonic	c.2308C>T	p.Gln770Ter	Nonsense	G=0.91, A=0.09
					chr1:179077299	T/C	<i>ABL2</i>	Exonic	c.3058A>G	p.Lys1020Glu	Missense	T=0.94, C=0.06
46	Poor	Yes	1	0	chr5:131944891	A/G	<i>RAD50</i>	Exonic	c.2912A>G	p.Asp971Gly	Missense	A=0.95, G=0.05
					chr12:56493991	A/G	<i>ERBB3</i>	Exonic	c.3163A>G	p.Met1055Val	Missense	A=0.11, G=0.89
					chr19:45855906	G/A	<i>ERCC2</i>	Exonic	c.1904C>T	p.Ala635Val	Missense	G=0.95, A=0.05
33	Poor	No	3	2	chr5:256478	C/G	<i>SDHA</i>	Exonic	c.1938C>G	p.Asp646Glu	Missense	C=0.89, G=0.11
83	Poor	No	2	1	chr19:3110167	A/G	<i>GNAI1</i>	Exonic	c.157A>G	p.Ser53Gly	Missense	A=0.92, G=0.08
					chr8:71040700	T/C	<i>NCOA2</i>	Exonic	c.3649A>G	p.Asn1217Asp	Missense	T=0.77, C=0.23
91	Poor	No	1	1	chr1:43812541	C/T	<i>MPL</i>	Exonic	c.1244C>T	p.Ser415Phe	Missense	C=0.85, T=0.15
21	Poor	No	1	1	chr14:95590549	C/T	<i>DICER1</i>	Exonic	c.1360G>A	p.Ala454Thr	Missense	C=0.52, T=0.48
36	Poor	No	1	1	chr13:29041176	A/C	<i>FLT1</i>	Exonic	c.252T>G	p.Asn84Lys	Missense	A=0.95, C=0.05
92	Poor	No	1	1	chr17:17124928	C/T	<i>FLCN</i>	Exonic	c.794G>A	p.Cys265Tyr	Missense	C=0.93, T=0.07
81	Good	No	4	4	chr6:117715816	A/C	<i>ROS1</i>	Exonic	c.942T>G	p.His314Gln	Missense	A=0.95, C=0.05
					chr18:59174619	G/A	<i>CDH20</i>	Exonic	c.843G>A	p.Met281Ile	Missense	G=0.90, A=0.10
39	Good	No	2	2	chr22:23524184	C/T	<i>BCR</i>	Exonic	c.1037C>T	p.Ser346Phe	Missense	C=0.95, T=0.05
					chr22:36684943	C/T	<i>MYH9</i>	Exonic	c.4600G>A	p.Val1534Met	Missense	C=0.95, T=0.05
76	Good	No	3	3	chr2:24933819	A/G	<i>NCOA1</i>	Exonic	c.2438A>G	p.Gln813Arg	Missense	A=0.72, G=0.28
					chr22:36684943	C/T	<i>MYH9</i>	Exonic	c.4600G>A	p.Val1534Met	Missense	C=0.94, T=0.06
23	Good	No	1	1	chr1:18960888	A/G	<i>PAX7</i>	Exonic	c.177A>G	p.Ile59Met	Missense	A=0.89, G=0.11
					chr4:55948732	C/G	<i>KDR</i>	Exonic	c.3733G>C	p.Glu1245Gln	Missense	C=0.68, G=0.32
62	Good	No	3	2	chr4:55946328	C/G	<i>KDR</i>	Exonic	c.3851G>C	p.Gly1284Ala	Missense	C=0.67, G=0.33
					chr15:57545527	T/A	<i>TCF12</i>	Exonic	c.1328T>A	p.Val443Glu	Missense	T=0.72, A=0.28
79	Good	No	2	2	chr10:123244924	T/C	<i>FGFR2</i>	Exonic	c.2183A>G	p.Asn728Ser	Missense	T=0.90, C=0.10
					chr19:30312940	C/G	<i>CCNE1</i>	Exonic	c.743C>G	p.Ser248Cys	Missense	C=0.91, G=0.09
82	Good	No	2	2	chr6:93982134	G/A	<i>EPHA7</i>	Exonic	c.1331C>T	p.Ser444Leu	Missense	G=0.94, A=0.06
					chr8:41798593	G/T	<i>KAT6A</i>	Exonic	c.2806C>A	p.Pro936Thr	Missense	G=0.87, T=0.13
82	Good	No	2	2	chr17:7579373	C/A	<i>TP53</i>	Exonic	c.314G>T	p.Gly105Val	Missense	C=0.18, A=0.82
					chrX:48896771	T/C	<i>TFE3</i>	Exonic	c.395A>G	p.Glu132Gly	Missense	T=0.95, C=0.05

ChOS, Chiba osteosarcoma; SNV, single-nucleotide variant.